

### Amendments to the Specification

Kindly replace the two paragraphs beginning on page 4, line 14, with the following:

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192) follows the lead of Cederholm-Williams by also teaching a method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which the fibrinogen in the plasma is first precipitated to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds times of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes.

These clotting speeds times are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Kindly replace the paragraph beginning on page 11, line 23, with the following:

Figure 8 is a chart describing the contaminating proteins removed from the enriched thrombin fraction after mixture with EtOH, (13.6% in final volume) and CaCl<sub>2</sub> (0.23 0.023  $\mu$ M in final volume) and filtered for particulate matter.

Kindly replace the paragraph beginning on page 12, line 8, with the following:

Figure 12 is a chart illustrating thrombin (Bovine) concentrations (activity) as it relates to speed time of clotting.

Kindly replace the paragraph beginning on page 14, line 8, with the following:

Referring to figure 3, the valve 24 is reoriented so that access can be gained between the mixing syringe 26 and the reagents found in ampoules 32, 34, each of which

are operatively connected to the manifold 22 via a Y coupling 36 shown in figure 1. Access to the interior of either ampoule 32 or 34 can be had by squeezing the ampoule to rupture a frangible diaphragm. Alternatively, the intake 38 which receives the ampoule can be provided with a hollow spike which penetrates the diaphragm. In either event, the contents of both of the ampoules 32, 34 are received in the mixing syringe 26 by further retraction of the plunger 28 along the arrow A shown in figure 3. A first ampoule 32 is preferably provided with 2 mL of ethanol providing an EtOH concentration in the final volume of 13.6% and the second ampoule 34 is preferably provided with 1 mL calcium chloride providing a concentration in the final volume of ~~.023~~ 0.023  $\mu$ M. Alternatively, these reagents contained within the two ampoules 32, 34 can be premixed into a single ampoule and dispensed simultaneously. In one form of the invention, it is possible to introduce the ethanol first, then agitate the mixing syringe 26 and then follow with the calcium chloride, but the introduction of both simultaneously to the plasma are optimally combined, followed by brief agitation.

Kindly replace the two paragraphs beginning on page 16, line 4, with the following:

Turning to figure 5, a graph is shown which illustrates how ethanol concentrations alter the life span of fast clotting thrombin where the calcium chloride content is held constant at ~~.023~~ 0.023  $\mu$ M. Note that at approximately 13.6% ethanol, its life span is shown to have been optimized and extend at least 240 minutes while its clotting time is substantially constant at under 5 seconds. The range between 8% and 18%, however, has utility.

Figure 6 varies the calcium chloride concentration in the thrombin while the ethanol is held constant at 13.6%. As shown, the thrombin life span where the final calcium chloride concentration is at ~~.023~~ 0.023  $\mu$ M (250mM) of 250mM calcium chloride appears optimized and extends to 360 minutes while maintaining a clot time under 5 seconds. The range of final calcium chloride concentration between ~~.011~~ 0.011

$\mu\text{M}$  (125 mM) of 125 mM and .045 0.045  $\mu\text{M}$  (500 mM) of 500 mM, however, has utility.

Kindly replace the paragraph beginning on page 16, line 20, with the following:

Figure 8 reflects the effect of using ethanol at 13.6% and calcium chloride at .023 0.023  $\mu\text{M}$  to reduce proteins which alter the clot time of the thrombin as compared to the original plasma. As can be seen in this graph, the major interfering proteins are so efficiently removed, that the clotting time of the thrombin is not only enhanced, but held substantially stable and constant.

Kindly replace the paragraphs beginning on page 17, line 1, with the following:

Figure 9 shows in greater detail than that which is shown in figures 5 and 6 regarding the measured clot time as a function of life span for the optimized thrombin preparation, having been treated by 13.6% ethanol and .023 0.023  $\mu\text{M}$  calcium chloride. As shown, the life span extends to 360 minutes and the clot time varies from 3 to 4 seconds.

Figure 10 shows the effect of saline solution on the thrombin preparation optimized as in figure 9 with an ethanol concentration of 13.6% and a calcium chloride concentration of .023 0.023  $\mu\text{M}$  as a function of life span. When the thrombin has been diluted 1 to 1.5 with saline, the clot time has been extended from just above 20 seconds to just less than 30 seconds, and has a life span of up to 150 minutes.

Kindly replace the paragraph beginning on page 17, line 11, with the following:

Referring to figure 11, there shown is the benefit in allowing the thrombin contained in the mixing syringe 26 to reside therein after agitation for almost 20 minutes in order to assure the effectiveness of the filtration step in removing particulate matter for subsequent utilization. The time span for conversion conversion and activation allows

enough particulate matter to be removed by the filter to optimize the use of the thrombin later in an narrow orificed dispenser, such as a sprayer or expressing through a thin tube.